

Divisional of 09/708,352  
Attorney Docket Number 12780/101

**CLAIM AMENDMENTS**

This listing of claims will replace all prior versions and listings of claims in the application:

1. (currently amended) A vaccine which is protective against *Mycoplasma bovis* clinical disease in a bovine species comprising at least one inactivated or attenuated *Mycoplasma bovis* biotype, an adjuvant, and a pharmaceutically acceptable excipient, and wherein the vaccine adjuvant does not include saponin and the clinical disease includes respiratory pneumonia.
2. canceled
3. (currently amended) The vaccine of claim 1, wherein the *Mycoplasma bovis* biotype is inactivated and the amount of each inactivated biotype is at least  $10^8$  *M. bovis* cells.
4. (currently amended) The vaccine of claim 1, wherein the *Mycoplasma bovis* biotype is attenuated and the amount of each attenuated biotype is at least  $10^5$  *M. bovis* cells.
5. (currently amended) A vaccine which is protective against *Mycoplasma bovis* clinical disease in a bovine species comprising at least one inactivated or attenuated *Mycoplasma bovis* biotype, an adjuvant, and a pharmaceutically acceptable excipient, wherein at least one of the inactivated or attenuated *Mycoplasma bovis* biotypes is selected from the group consisting of biotype A, biotype B and Biotype C, and wherein the vaccine adjuvant does not include saponin.
6. (currently amended) The vaccine of claim 5, wherein the *Mycoplasma bovis* biotype is inactivated and the amount of each selected inactivated *Mycoplasma bovis* biotype is at least  $10^8$  *M. bovis* cells.

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7. (currently amended) The vaccine of claim 5, wherein the *Mycoplasma bovis* biotype is attenuated and the amount of each selected attenuated *Mycoplasma bovis* biotype is at least  $10^5$  *M. bovis* cells.
8. (currently amended) The vaccine of claim 1, wherein the A vaccine which is protective against *Mycoplasma bovis* clinical disease in a bovine species comprising at least two inactivated or attenuated *Mycoplasma bovis* biotypes and a pharmaceutically acceptable excipient.
9. (original) The vaccine of claim 8, further comprising a suitable adjuvant.
10. (currently amended) The vaccine of claim 8, wherein the *Mycoplasma bovis* biotype is inactivated and the amount of each inactivated biotype is at least  $10^8$  *M. bovis* cells.
11. (currently amended) The vaccine of claim 8, wherein the *Mycoplasma bovis* biotype is attenuated and the amount of each attenuated biotype is at least  $10^5$  *M. bovis* cells.
12. (currently amended) The vaccine of claim 8, wherein the *Mycoplasma bovis* biotype is biotypes are selected from the group consisting of biotype A, biotype B and biotype C.
- 13-28. (canceled)
29. (new) A vaccine which is protective against *Mycoplasma bovis* mastitis in a bovine species comprising at least one inactivated or attenuated *Mycoplasma bovis* biotype and a pharmaceutically acceptable excipient.
30. (new) The vaccine of claim 29, where the vaccine is protective against *Mycoplasma bovis* mastitis in a bovine species following systemic administration.

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31. (new) The vaccine of claim 30, comprising at least two inactivated *Mycoplasma bovis* biotypes.
32. (new) The vaccine of claim 31, wherein the vaccine includes at least one inactivated *Mycoplasma bovis* biotype selected from the group consisting of biotype A, biotype B and biotype C.
33. (new) The vaccine of claim 31 wherein the vaccine contains approximately  $10^8$  cells of each biotype in a volume of 2-5 milliliters.
34. (new) The vaccine of claim 8 wherein the at least two inactivated or attenuated *Mycoplasma bovis* biotypes are genetically different as determined by an analysis of DNA or RNA from the biotypes.
35. (new) The vaccine of claim 34 wherein the analysis is by PCR fingerprinting, analysis of ribosomal RNA, or analysis of DNA polymorphisms.
36. (new) The vaccine of claim 35 wherein the analysis is by PCR fingerprinting.
37. (new) The vaccine of claim 36 wherein the PCR fingerprinting uses arbitrarily chosen primers.
38. (new) The vaccine of claim 37 wherein the PCR fingerprinting uses as primers 5' NNN NCG NCG NCA TCN GGC 3' (SEQ ID NO:1) and 5' NCG NCT TAT CNG GCC TAC 3' (SEQ ID NO:2).
39. (new) The vaccine of claim 8 wherein the at least two *Mycoplasma bovis* biotypes have been identified as being different biotypes by a process comprising:
  - (a) isolating DNA from the biotypes;
  - (b) amplifying the DNA by PCR;

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(c) separating the amplified DNA by gel electrophoresis; and

(d) comparing the resulting patterns from the gel electrophoresis to identify the different biotypes.

40. (new) The vaccine of claim 30 wherein, when the vaccine is administered to a plurality of cows in a herd of cows, the incidence of mastitis caused by *Mycoplasma bovis* in the herd before administering is greater than the incidence of mastitis caused by *Mycoplasma bovis* in the herd after administering.

41. (new) The vaccine of claim 40 wherein the vaccine is administered to at least about 50% of the herd.

42. (new) The vaccine of claim 41 where the vaccine is administered together with an adjuvant.

43. (new) The vaccine of claim 42 wherein the adjuvant is an aluminum hydroxide-oil emulsion; a mineral, vegetable, or fish oil-water emulsion; a water-oil-water emulsion; incomplete Freund's adjuvant; *E. coli* J5; dextran sulfate; iron oxide; sodium alginate; Bacto-Adjuvant; a synthetic polymer; Carbopol; a poly-amino acid; a co-polymer of amino acids; saponin; carrageenan; REGRESSIN®; N, N-di octadecyl-N'-N'-bis(2-hydroxyethyl) propanediamine; a long chain polydispersed  $\beta$ (1,4) linked mannan polymer interspersed with O-acetylated groups; deproteinized cell wall extracts from a non-pathogenic strain of *Mycobacterium*; mannite monooleate; paraffin oil; or muramyl dipeptide.

44. (new) The vaccine of claim 30 where the *Mycoplasma bovis* biotype is inactivated and has been inactivated by treatment with: formalin, azide, freeze-thawing, sonication, heat, sudden pressure drop, detergent, lysozyme, phenol, proteolytic enzymes,  $\beta$ -propiolactone, Thimerosal, or binary ethyleneimine.

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45. (new) The vaccine of claim 44 where the *Mycoplasma bovis* biotype has been inactivated by treatment with  $\beta$ -propiolactone.
46. (new) The vaccine of claim 31 wherein the at least two inactivated *Mycoplasma bovis* biotypes are genetically different as determined by an analysis of DNA or RNA from the biotypes.
47. (new) The vaccine of claim 46 wherein the analysis is by PCR fingerprinting, analysis of ribosomal RNA, or analysis of DNA polymorphisms.
48. (new) The vaccine of claim 47 wherein the analysis is by PCR fingerprinting.
49. (new) The vaccine of claim 48 wherein the PCR fingerprinting uses arbitrarily chosen primers.
50. (new) The vaccine of claim 49 wherein the PCR fingerprinting uses as primers 5' NNN NCG NCG NCA TCN GGC 3' (SEQ ID NO:1) and 5' NCG NCT TAT CNG GCC TAC 3' (SEQ ID NO:2).
51. (new) The vaccine of claim 31 wherein the at least two *Mycoplasma bovis* biotypes have been identified as being different biotypes by a process comprising:
  - (a) isolating DNA from the biotypes;
  - (b) amplifying the DNA by PCR;
  - (c) separating the amplified DNA by gel electrophoresis; and
  - (d) comparing the resulting patterns from the gel electrophoresis to identify the different biotypes.
52. (new) A whole-cell vaccine which is protective against *Mycoplasma bovis* clinical disease in a bovine species comprising at least one inactivated or attenuated *Mycoplasma bovis* biotype and an adjuvant selected from the group consisting of: an aluminum hydroxide-oil

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emulsion; a mineral, vegetable, or fish oil-water emulsion; a water-oil-water emulsion; *E. coli* JS; dextran sulfate; iron oxide; sodium alginate; Bacto-Adjuvant; a synthetic polymer; Carbopol; a poly-amino acid; a co-polymer of amino acids; carrageenan; REGRESSIN®; N, N-dioctadecyl-N'-N'-bis(2-hydroxyethyl) propanediamine; a long chain polydispersed  $\beta$ (1,4) linked mannan polymer interspersed with O-acetylated groups; deproteinized cell wall extracts from a non-pathogenic strain of *Mycobacterium*; mannite monooleate; and paraffin oil.

53. (new) The vaccine of claim 1, wherein the *Mycoplasma bovis* biotype is inactivated.

54. (new) The vaccine of claim 5, wherein the *Mycoplasma bovis* biotype is inactivated.

55. (new) The vaccine of claim 52, wherein the *Mycoplasma bovis* biotype is inactivated.

56. (new) A vaccine which is protective against *Mycoplasma bovis* clinical disease in a bovine species comprising at least one attenuated *Mycoplasma bovis* biotype and a pharmaceutically acceptable excipient, wherein the clinical disease includes respiratory pneumonia.